

D-Fructose/D-Glucose, UV method

Catalogue number: AK00041, 110 tests (manual) / 1100 tests (microplate)

Application

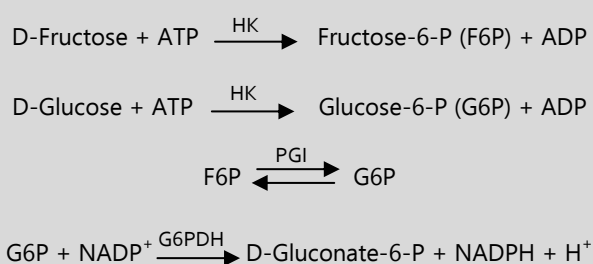
This rapid and simple specific enzymatic method is used for the simultaneous determination of D-fructose and D-glucose in foodstuffs, pharmaceuticals, cosmetics and biological samples.

The analysis of these sugars can also be performed separately. This kit can be used for the auto-analysis of total reducing sugars (for further information on literature or analytical kits, please contact NZYTech).

Introduction

D-Fructose and D-glucose occur widely in plant organisms. In foods, they occur mainly in honey, wine and beer, and a range of solid foodstuffs such as bread and pastries, chocolate and candies. In the wine industry, the content of D-fructose and D-glucose (total reducing sugars) is one of the key quality parameters; it represents the amount of sugar available for yeast fermentation.

Principle



The amount of NADPH formed through the combined action of hexokinase (HK), phosphoglucose isomerase (PGI) and glucose-6-P dehydrogenase (G6PDH), measured at 340 nm, is stoichiometric with the amount of D-fructose and D-glucose in sample volume.

Specificity

This method is specific for D-fructose and D-glucose.

Sensitivity and detection limit

The sensitivity of the assay is based on 0.010 AU and a sample volume of 2.00 mL. This corresponds to a D-fructose and D-glucose concentration of 0.33 mg/L sample solution when measured at 340 nm. The detection limit of 0.66 mg/L is derived from the absorbance difference of 0.020 (340 nm) and a maximum sample volume of 2.00 mL.

Linearity and precision

Linearity of the determination exists from 4 to 80 µg D-fructose and D-glucose per assay ($v = 2.00$ mL). In a double assay using one sample solution, a difference of 0.005 to 0.010 absorbance units may occur, which corresponds to a D-fructose and D-glucose concentration of approx. 0.17 to 0.33 mg/L ($v = 2.00$ mL). The coefficient of variation is approx. 1 to 2 %.

Kit composition

Solution 1. Imidazole buffer (25 mL, 2 M, pH 7.6) plus MgCl_2 (100 mM) and sodium azide (0.02 % w/v) as a preservative. Stable for 2 years at 4 °C.

Solution 2. NADP^+ (250 mg) plus ATP (500 mg) and PVP (120 mg). Stable for 2 years at -20 °C.

Dissolve in 12 mL of distilled water, divide into appropriately sized aliquots and store in PP tubes at -20 °C between use (stable for 2 years) and keep cool during use.

Suspension 3. Hexokinase (EC 2.7.1.1; 425 U/mL) and Glucose-6-P dehydrogenase (EC 1.1.1.49; 212 U/mL) in 3.2 M ammonium sulphate (2.25 mL). Stable for 2 years at 4 °C. Swirl bottle before use.

Suspension 4. Phosphoglucose isomerase (EC 5.3.1.9; 1000 U/mL) in 3.2 M ammonium sulphate (2.25 mL). Stable for 2 years at 4 °C. Swirl bottle before use.

Solution 5. D-Fructose/D-glucose standard solution (5 mL, 0.20 mg/mL of each sugar). Stable for 2 years at room temperature. This standard solution can be used when there is some doubt about the method accuracy.

Safety

Reagents that are used in the determination of D-fructose plus D-glucose are not hazardous materials (see Hazardous

Substances Regulations). The general safety measures that apply to all chemical substances should be followed.

Procedure (endpoint analysis)

Wavelength: 340 nm

Cuvette: 1 cm light path (glass or plastic)

Temperature: ~ 25 °C

Final volume: 2.32 mL (D-glucose); 2.34 mL (D-fructose)

Sample solution: 4-80 µg of D-glucose plus D-fructose per cuvette (in 0.10-2.00 mL sample volume)

Read against air (without a cuvette in the light path) or against water

Pipette into cuvettes (mL)	Blank	Sample
Distilled water (at ~25 °C)	2.10	2.00
Sample	-	0.10
Solution 1 (imidazole buffer)	0.10	0.10
Solution 2 (NADP ⁺ +ATP)	0.10	0.10
Mix, measure the absorbance of the above solutions (A1) after approx. 3 min and start the reaction by addition of		
Suspension 3 (HK+G6PDH)	0.02	0.02
Mix, measure the absorbance of the above solutions (A2) at the end of the reaction (approx. 5 min)*		
Suspension 4 (PGI)	0.02	0.02
Mix, measure the absorbance of the above solutions (A3) at the end of the reaction (approx. 8-10 min)*		

Mixtures can be obtained with a plastic spatula or by gentle inversion after sealing with a cuvette cap or Parafilm®.

* if necessary, continue to read the absorbance at 2 min intervals until the reaction ends.

Calculation

Determine the absorbances difference for both blank and sample (A2-A1). Subtract the absorbances difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{D\text{-glucose}}$. Determine the absorbance difference for both blank and sample (A3-A2). Subtract the absorbances difference of the blank from the absorbances difference of the sample, thereby obtaining $\Delta A_{D\text{-fructose}}$. The concentration of D-fructose (g/L) and D-glucose (g/L), based on the Abs of NADH at 340 nm ($6300 \text{ L}\times\text{mol}^{-1}\times\text{cm}^{-1}$), are calculated as follows:

$$C(D\text{-Fructose}) = 0.6692 \times \Delta A_{D\text{-Fructose}} \quad [\text{g/L}]$$

$$C(D\text{-Glucose}) = 0.6634 \times \Delta A_{D\text{-Glucose}} \quad [\text{g/L}]$$

If the sample has been diluted or a different sample volume was used during the reaction, the result must be multiplied by the corresponding dilution/concentration factor.

Alternative procedures (micro-volumes)

Although this kit has been developed to work in cuvettes, it can be easily adapted for use in 96-well microplates or in auto-analysers. Basically, the assay volumes for the cuvette format have to be reduced approximately 10-fold for use in microplate format or in auto-analyser format. However, when using these micro-volume formats, you must be aware that the radiation pathlength is usually smaller than 1 cm, which is the standard cuvettes pathlength. Thus, to perform the calculation of the amount of analyte in the samples follow one of the three possible strategies described in the "Alternative Procedures Brochure", available on the NZYTech website.

Interferences

If the conversion of D-fructose and D-glucose completes within the time specified in the assay (approx. 10 min), we can be generally concluded that no interference has occurred. However, an internal standard should be included during sample analysis if the presence of interfering substances is suspected. A quantitative recovery of this standard should be expected. Identification of losses in sample handling and extraction may be identified by performing recovery experiments, i.e., by adding D-fructose and D-glucose to the sample in the initial extraction steps.

General information on sample preparation

The amount of D-fructose plus D-glucose present in the cuvette should range between 4 and 80 µg. Thus, if a sample volume of 0.10 mL is used the sample solution must be diluted to yield a sugar concentration between 40 and 800 mg/L. However, the sample volume can range from 0.10 to 2.00 mL, by replacing water (analytes range from 2 to 800 mg/L).

To implement this assay use clear, colourless and practically neutral liquid samples directly, or after dilution; filter turbid solutions; degas samples containing carbon dioxide (e.g. by filtration); adjust acid samples, which are used undiluted for the assay, to pH 8 by adding sodium or potassium hydroxide solution; adjust acid and weakly coloured samples to pH 8 and incubate for approx. 15 min; measure "coloured" samples (if necessary adjusted to pH 8) against a sample blank; treat "strongly coloured" samples that are used undiluted or with a higher sample volume with PVPP; crush or homogenize solid or semi-solid samples, extract with water or dissolve; extract samples containing fat with hot water.

Examples of sample preparation

Determination of D-fructose and D-glucose in red and white wine

Determination of D-fructose and D-glucose concentration of white and red wine can be usually performed without any sample treatment, except dilution. Should a red wine require decolourisation, proceed as described previously. Typically, a

dilution of 1:10 and a sample volume of 0.1 mL are satisfactory.

Determination of D-glucose and D-fructose in honey

After stirring the honey sample thoroughly with a spatula, transfer approx. 5-10 g of the viscous or crystalline material to a beaker and heat for 5 min at approx. 60 °C (there is no need to stir liquid honey). After cooling, pour approx. 1 g of the liquid sample accurately weighed, into a 100 mL volumetric flask. Dissolve initially with only a small volume of distilled water and then dilute to the mark and mix. Typically, a dilution of 1:10 and a sample volume of 0.1 mL is satisfactory.

Determination of D-glucose and D-fructose in solid foodstuffs

Plant materials should be milled to pass a 0.5 mm screen. Homogenise solid foodstuffs such as bread, pastries, chocolate confectionery or candy in a mixer, meat grinder or mortar. A representative sample should be weighed and extracted with water (heated to 60 °C, if necessary). Quantitatively transfer to a volumetric flask and dilute. Mix, filter and use the appropriately diluted, clear solution for the assay.

References

Kunst, A., Draeger, B. & Ziegenhorn, J. (1988). D-Glucose. In: Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 3rd ed., Vol.VI, pp. 163-172, VCH Publishers (UK) Ltd., Cambridge, UK.

Recommendations

This method is recommended/approved by the:

- European Commission Regulation (analysis of wine);
- Association of Official Analytical Chemists (AOAC) (analysis of wine);
- European, Dutch, French, German and Russian standards (EN, NEN, NF, DIN, GOST);
- International Wine Office (OIV), International Federation of Fruit Juice Producers (IFU), Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Economic Community (A.I.J.N.), and Mitteleuropäische Brautechnische Analysenkommission (MEBAK) (Central European Commission for Brewing Technology).

Released 12/12

Certificate of Analysis

Test	Criteria	Result
Test Performance	Reaction completed within time stated	Meets specification
	Target value for recommended standard material +/- 10%	Meets specification
Blank reaction absorbance	+/- 10% of the blank value	Meets specification

Approved by:



José Prates
Senior Manager, Quality Systems

Please enquire info@nzytech.com to obtain any additional information about this kit, including additional specific applications.



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